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Cloning, Sequence, and Properties of the Soluble Pyridine Nucleotide Transhydrogenase of *Pseudomonas fluorescens*

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The gene encoding the soluble pyridine nucleotide transhydrogenase (STH) of *Pseudomonas fluorescens* was cloned and expressed in *Escherichia coli*. STH is related to the flavoprotein disulfide oxidoreductases but lacks one of the conserved redox-active cysteine residues. The gene is highly similar to an *E. coli* gene of unknown function.

Pyridine nucleotide transhydrogenases catalyze the transfer of reducing equivalents between NAD and NADP pools. A membrane-bound, proton-pumping transhydrogenase, specific for the 4A proton of NADH and the 4B proton of NADPH, occurs in mitochondria and in some bacteria, such as *Escherichia coli*, and has been studied in some detail (3, 8). This enzyme couples proton import with oxidation of NADH and reduction of NADP⁺, and its physiological role is believed to be production of NADPH for reductive biosyntheses. Less well-known is a soluble transhydrogenase (STH) reported to occur in *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Azotobacter vinelandii* (13). This enzyme is a flavoprotein specific for the 4B protons of both NADH and NADPH. STH is not energy dependent but is strongly inhibited by NADP⁺, suggesting that its physiological role is the conversion of NADPH generated by peripheral catabolic pathways in these bacteria to NADH, which can be oxidized for energy generation (16). STH is remarkable for its formation of large polymers. The subunit M_r is approximately 54,000, whereas the minimal active form of the *P. aeruginosa* enzyme has an M_r of approximately 1.6 million (18). This minimal form further aggregates on isolation to form filaments of lengths exceeding 500 nm (9). The enzyme from *A. vinelandii* displays similar behavior, although the structure of the filaments appears to be different (15). STH also shows interesting kinetic behavior, with activity strongly activated by NADPH and 2'-AMP and inhibited by NADP⁺ (19). The presence of Ca²⁺ favors activation and reduces inhibition.

To gain some insight into the structural basis for the aggregation and regulation of this unusual enzyme, we sought to clone the gene encoding STH from *P. fluorescens* NCIMB9815, a close relative of *P. aeruginosa*.

Purification of STH from *P. fluorescens*. STH activity was assayed by following the reduction of thionicotinamide adenine dinucleotide (tNAD⁺) at 400 nm in a reaction mixture consisting of 0.1 mM NADPH and 0.1 mM tNAD⁺ (Sigma Chemical Co.) in 50 mM Tris-HCl buffer (pH 7.0). The molar extinction coefficient of tNADH at 400 nm was taken as 11,300 liters mol⁻¹ cm⁻¹ (2). One unit of enzyme activity was defined as that amount of activity reducing 1 μ mol of tNAD⁺ per min under these conditions.

STH was purified from cells of *P. fluorescens* NCIMB9815

according to a modification of the method of Höjeberg et al. (7). Cells were grown to stationary phase in 1 liter of SOB medium (14). The cells were harvested by centrifugation (5,000 \times g for 15 min) and resuspended in 20 ml of buffer A (50 mM Tris-HCl [pH 7.0] with 2 mM dithiothreitol). The cells were then disrupted by sonication (25 bursts of 5 s at 12 μ m separated by 30-s pauses for cooling in an ice-water bath) with an MSE Soniprep 150. Cell debris was removed by centrifugation (25,000 \times g for 10 min). The extract contained 93 U of STH activity at a specific activity of 0.19 U/mg.

STH was purified by using a column with an inner diameter of 1 cm, packed with 6 ml of adenosine-2',5'-diphosphate agarose (packed height, 7.6 cm) (Sigma Chemical Co.). The column was operated at 12 ml/h during loading and at 24 ml/h during washing. All procedures were performed at 4°C, and all buffers contained 2 mM dithiothreitol. After equilibration of the column with 5 mM CaCl₂ in buffer A, crude extract (20 ml), to which CaCl₂ had been added to a final concentration of 5 mM, was loaded onto the column. The column was then washed with 90 ml of 0.4 M NaCl–5 mM CaCl₂ in buffer A, followed by 24 ml of 0.7 M NaCl–5 mM CaCl₂ in buffer A. Bound STH was eluted with 50 mM Tris-HCl (pH 8.9) containing 0.4 M NaCl. Active fractions (15 ml) were pooled. The pooled product was concentrated by ultrafiltration with an Amicon 8050 ultrafiltration cell fitted with a membrane with a nominal M_r cutoff of 10,000 and was then diafiltered with buffer A to reduce the pH and salt concentration. The final volume was 1.5 ml. This material contained 62 U of STH activity at a specific activity of 140 U/mg.

This product was then applied to a gel filtration column with an inner diameter of 1.6 cm, packed with 150 ml of Sephacryl S-300 (packed height, 75 cm) (Pharmacia) equilibrated with buffer A. The column was operated at 8 ml/h. Active fractions (16 ml) were pooled and concentrated by ultrafiltration as described above to a final volume of 1 ml. The product contained 26 U of STH activity at a specific activity of 310 U/mg, an overall 1,630-fold purification.

Prior to analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the sample was further concentrated by freeze-drying and resuspension in a small volume of buffer A. The reconstituted material was not active. SDS-PAGE showed a single protein band with an apparent M_r of approximately 54,000, consistent with the value reported for the enzyme from *P. aeruginosa* (18).

Cloning of the *sth* gene. The N-terminal sequence of STH was determined by automated Edman degradation and was found to be A-V-Y-N-Y-D-V-V-V-L-G-S-(G/V)-P-A-G-E-(G/

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208	ACGGGCGGTG AAACGTAACG AGTGTCTGTG AGTAAAGGCA AAACCTGGCTA	-159	580	TTC AGC GGC CTG GGT GTG CTG GTC GAG CTG GTC AAC CGC GAC	624
158	CGGAACGCGA CCAAGGGTTA ATGTGCGCGG CQTGTAGCCT TATATAGACT	-109		Phe Ser Gly Leu Gly Val Leu Val Glu Leu Val Asp Asn Arg Asp	
108	GTGCCCCGGT TCAACACACT TGAGCCAAAT TGTCTTCAT GACCGCTGGG	-59		190 195 200	
	SacII		625	CAG TTG CTG AGC TTC CTC GAC TCG GAA ATC TCC CAG GCG TTG AGC	669
-58	CGCGCATGA TTTAGCCAGG CGTCTCAACG TACGCTGGC CTGTTTGTAG	-9		Gln Val Leu Ser Phe Leu Asp Ser Glu Ile Ser Glu Ala Leu Ser	
				205 210 215	
-8	GAGTACGC ATG GCT GTC TAC AAC TAC GAC GTG GTG GTA CTG GGT TCC	39	670	TAC CAC TTC AGC AAC AAC AAC ATC ACT GTG CCC CAT AAC GAA GAG	714
	Met Ala Val Tyr Asn Tyr Asp Val Val Val Leu Gly Ser			Tyr His Phe Ser Asn Asn Asn Ile Thr Val Arg His Asn Glu Glu	
	-1 1 5 10			220 225	
40	GGC CCG GCT GGA GAA GGT GCG GCG ATG AAC GCC GCG AAG GCA GGG	84	715	TAC GAT CGG GTC GAA GGC CTG GAC AAC GGG GTG ATC CTG CAC CTC	759
	Gly Pro Ala Gly Glu Gly Ala Ala Met Asn Ala Ala Lys Ala Gly			Tyr Asp Arg Val Glu Gly Leu Asp Asn Glu Val Ile Leu His Leu	
	15 20 25			235 240 245	
85	CGC AAG GTG GCG ATG GTC GAT AGC CGT GCG CAG GTC GGC GGT AAC	129	760	AAG TCC GGC AAG AAG ATC AAG GCC GAC GCC TTG CTG TGG TGC AAC	804
	Arg Lys Val Ala Met Val Asp Ser Arg Arg Gln Val Gly Gly Asn			Lys Ser Gly Lys Lys Ile Lys Ala Asp Ala Leu Leu Trp Cys Asn	
	30 35 40			250 255 260	
130	TGC ACC CAC CTG GGT ACC ATC CCG TCC AAG GCA TTG CGT CAC TCC	174	805	GGT CGT ACC GGC AAC ACC GAC AAG CTG GGC ATG GAA AAC ATC GGG	849
	Cys Thr His Leu Gly Thr Ile Pro Ser Lys Ala Leu Arg His Ser			Gly Arg Thr Gly Asn Thr Asp Lys Leu Gly Met Glu Asn Ile Gly	
	45 50 55 60 65			265 270 275	
175	GTT CGC CAG ATC ATG CAG TTC AAC ACC AAC CCG ATG TTC CGG GCC	219	850	GTC AAG GTC AAC AGC CGT GGC CAG ATC GAG GTG GAC GAA AAC TAC	894
	Val Arg Gln Ile Met Gln Phe Asn Thr Asn Pro Met Phe Arg Ala			Val Lys Val Asn Ser Arg Gly Gln Ile Glu Val Asp Glu Asn Tyr	
	60 65 70			280 285 290	
220	ATT GGC GAG CCG GCG TGG TTC TCG TTC CCG GAT GTG TTT AAA AGC	264	895	CGC ACC TGT GTG ACC AAC ATC TAT GGC GCC GGT GAC GTG ATC GGC	939
	Ile Gly Glu Pro Arg Trp Phe Ser Phe Pro Asp Val Leu Lys Ser			Arg Thr Cys Val Thr Asn Ile Tyr Gly Ala Gly Asp Val Ile Gly	
	70 75 80			295 300 305	
265	GCT GAA AAA GTC ATC TCC AAG CAA GTC GCC TCG CGT ACC GGC TAC	309	940	TGG CCG AGC CTG CGC AGT GCC GCC CAT CAC GAG GGC CGT TCG GCC	984
	Ala Glu Lys Val Ile Ser Lys Gln Val Ala Ser Arg Thr Gly Tyr			Trp Pro Ser Leu Ala Ser Ala Ala His Asp Gln Gly Arg Ser Ala	
	85 90 95			310 315 320	
310	TAC GCC CGT AAC CGC GTC GAC CTG TTC TTC GGT ACC GGC AGC TTC	354	985	GCT GGC AGC ATC GTC GAC AAC GGC AGC TGG CGC TAT GTG AAC GAC	1029
	Tyr Ala Arg Asn Arg Val Asp Leu Phe Phe Gly Thr Gly Ser Phe			Ala Gly Ser Ile Val Asp Asn Gly Ser Trp Arg Tyr Val Asn Asp	
	100 105 110			325 330 335	
355	GCC GAC GAG CAA ACC CTC GAG GTG GTC TGC GCC AAT GGC GTG GTC	399	1030	GTA CCG ACC GGC ATC TAC ACG ATT CCG GAG ATC AGC TCG ATC GGC	1074
	Ala Asp Glu Gln Thr Val Glu Val Val Cys Ala Asn Gly Val Val			Val Pro Thr Gly Ile Tyr Thr Ile Pro Glu Ile Ser Ser Ile Gly	
	115 120 125			340 345 350	
400	GAG AAA CTG GTG GCC AAG CAC ATC ATC ATT GCC ACC GGC TCG CGC	444	1075	AAG AAC CAA CAC GAA CTG ACC AAG GCC AAG GTG CCT TAC GAA GTG	1119
	Glu Lys Leu Val Ala Lys His Ile Ile Ile Ala Thr Gly Ser Arg			Lys Asn Glu His Glu Leu Thr Lys Ala Lys Val Pro Tyr Glu Val	
	130 135 140			355 360 365	
445	CCG TAT CGC CCG GCG GAT ATC GAT TTC CAC CAC CCA CGT ATC TAC	489	1120	GGC AAG GCG TTC TTC AAG AGC ATG GCG CGT GCG CAG ATC GCC GGT	1164
	Pro Tyr Arg Pro Ala Asp Ile Asp Phe His His Pro Arg Ile Tyr			Gly Lys Ala Phe Phe Lys Ser Met Ala Arg Ala Gln Ile Ala Gly	
	145 150 155			370 375 380	
490	GAT AGC GAT ACC ATC CTC AGC CTG GGC CAC ACC CCA CGC AAA CTG	534	1165	GAG CCG CAA GGC ATG CTG AAG ATC CTG TTT CAC GCG GAG ACC CTG	1209
	Asp Ser Asp Thr Ile Leu Ser Leu Gly His Thr Pro Arg Lys Leu			Glu Pro Gln Gly Met Leu Lys Ile Leu Phe His Arg Glu Thr Leu	
	160 165 170			385 390 395	
535	ATC ATC TAT GGC GCC GCG GTC ATT GGC TGT GAA TAC GCC TCG ATC	579	1210	GAA GTC CTC GGC GTG CAT TGC TTC GGC TAC CAG GCT TCG GAG ATC	1254
	Ile Ile Tyr Gly Ala Gly Val Ile Gly Cys Glu Tyr Ala Ser Ile			Glu Val Leu Gly Val His Cys Phe Gly Tyr Gln Ala Ser Glu Ile	
	175 180 185			400 405 410	
			1255	GTG CAC ATC GGC CAG GCC ATC ATG AAC CAG CCG GGC GAG CAA AAT	1299
				Val His Ile Gly Gln Ala Ile Met Asn Gln Pro Gly Gln Asn	
				415 420 425	
			1300	ACC CTC AAG TAT TTC GTC AAC ACC ACC TTC AAC TAC CCG ACC ATG	1344
				Thr Leu Lys Tyr Phe Val Asn Thr Thr Phe Asn Tyr Pro Thr Met	
				430 435 440	
			1345	GCC GAA GCC TAT CGG GTA GCG GCC TAC GAT GGC CTC AAC CGG CTT	1389
				Ala Glu Ala Tyr Arg Val Ala Ala Tyr Asp Arg Gly Leu Asn Arg Leu	
				445 450 455	
			1390	TTT TGA GCGGCTCCGG CCGGTGGCCT GAGCCGCCG GGGAGACCGA TTTCAGTAA	1443
				Phe *	
				XhoI	
			1444	TTCTCGAG	

FIG. 1. Sequence of *sth* and deduced amino acid sequence of STH. Both strands were sequenced over the region shown. Nucleotides are numbered, starting with 1 for the A of the initiating ATG.

V)-A-A-M-N-A-A-(R/D); parentheses indicate uncertain assignments.

Based on the codon bias of *P. fluorescens* genes in the sequence databases, the following degenerate oligonucleotide was designed: AC-(C/G)AC-(C/G)AC-GTC-GTA-GTT-GTA-(C/G)AC-(G/C)GC (based on residues 1 to 9 of the N-terminal sequence).

Southern blotting and cloning procedures were performed according to standard methods (14). Southern blots of genomic DNA from *P. fluorescens* NCIMB9815 showed that this oligonucleotide bound most strongly to a 5.0-kb *Eco*RI fragment. This fragment was cloned in pBluescript SK+ in both orientations. The recombinant plasmids were designated pSTH-G1 and pSTH-G2. The gene *sth* was localized by restriction mapping of the insert followed by Southern blot analysis with the oligonucleotide probe. Sequencing indicated the presence of an open reading frame encoding a protein of the same N-terminal sequence as that determined for STH. Various subclones were prepared in pBluescript SK+ and sequenced by using vector-based primers. The sequence of *sth* and the deduced amino acid sequence of STH are shown in Fig. 1. The gene encodes a protein of 463 residues with an M_r of 50,876, as determined by the Genetics Computer Group (GCG) PEP-TIDESORT program (4), excluding the initiating methionine. This is slightly lower than the M_r expected from the migration position in SDS-PAGE.

FIG. 1—Continued.

Expression of STH in *E. coli*. Cell extracts prepared from saturated cultures of *E. coli* JM109/pSTH-G1 or -pSTH-G2 showed detectable STH activity, assayed by the reduction of tNAD⁺ in the presence of NADPH. A 1.5-kb *Sac*II/*Xho*I fragment from pSTH-G1 was subcloned in pBluescript SK+. This plasmid was designated pSTH1. In pSTH1, *sth* is in the correct orientation to be expressed from the *lac* promoter of pBluescript SK+. Cell extracts from saturated cultures of *E. coli* JM109/pSTH1 in the absence or presence of 0.4 mM IPTG (isopropyl- β -D-thiogalactopyranoside) showed transhydrogenase activity of 4.1 or 22.0 U/mg, respectively. Based on the specific activity of purified STH, it was estimated that in the latter case STH formed approximately 6% of soluble cell protein, approximately 100 times the level seen in *P. fluorescens*.

The recombinant STH was purified to apparent homogeneity in a single affinity chromatography step by using adenosine-2',5'-diphosphate agarose. Cell extract was prepared as described above from 1 liter of saturated culture of *E. coli* JM109/pSTH1 grown in the presence of 0.4 mM IPTG. Of the resulting 25 ml of cell extract, 5 ml, containing 2,140 U of STH

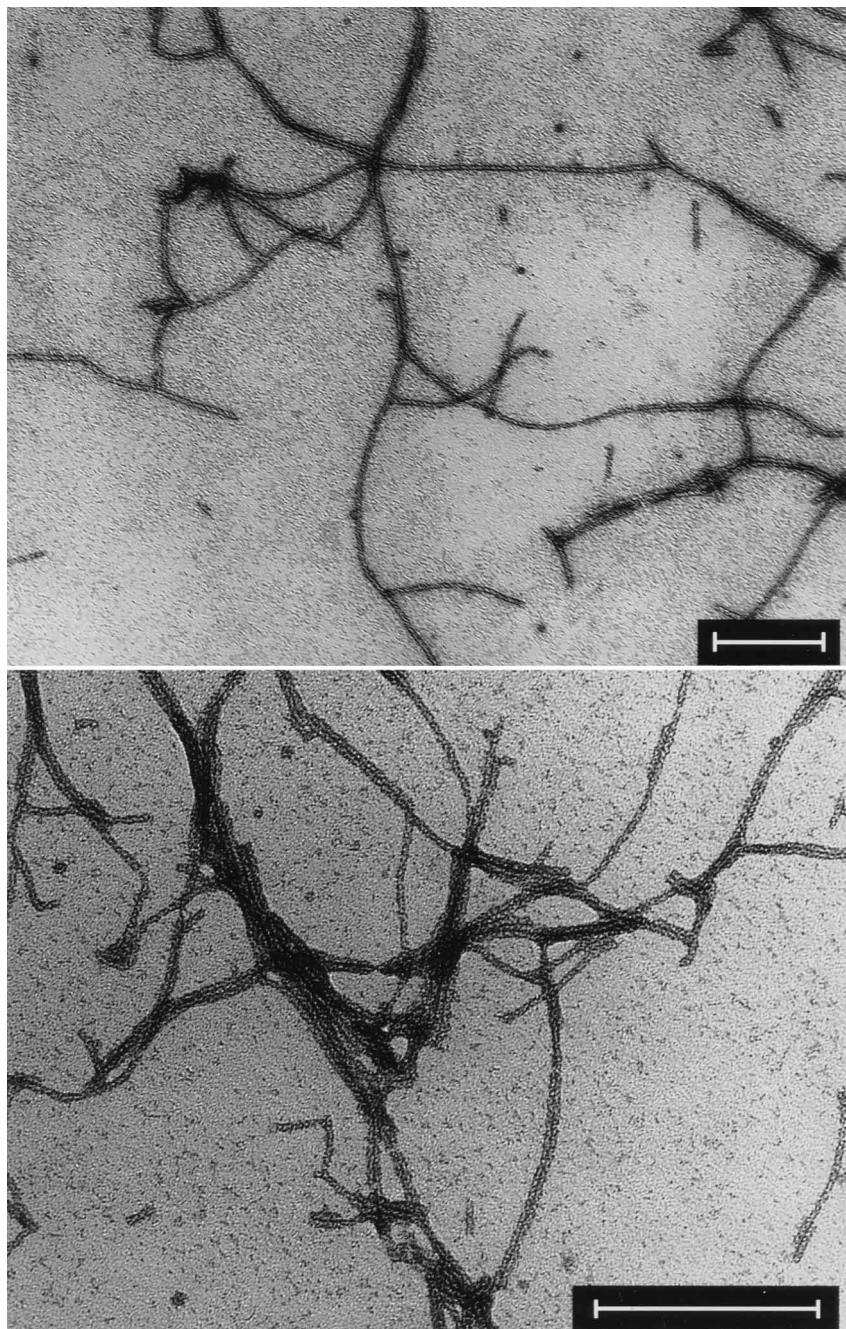


FIG. 2. Electron micrographs showing formation of filaments by purified STH. STH was adsorbed onto glow discharged carbon Formvar films from a 1.0-mg/ml solution in 50 mM Tris-HCl buffer (pH 7.0). Grids were then negatively stained with 1% (wt/vol) uranyl acetate and examined with a Philips CM100 electron microscope at 80 kV. Bars, 200 nm.

activity at a specific activity of 27 U/mg, was loaded onto a column packed with adenosine-2',5'-diphosphate agarose as described above. The column was washed with 35 ml of 0.7 M NaCl–5 mM CaCl_2 in buffer A. STH was then eluted with 0.4 M NaCl in 50 mM Tris-HCl (pH 8.9). The most active fractions, totalling 13 ml, were pooled, concentrated, and diafiltered as described above, except that a membrane with a nominal M_r cutoff of 300,000 was used. The product contained 900 U of STH activity at a specific activity of 300 U/mg. This material appeared to be homogeneous by SDS-PAGE; the gel filtration step was therefore omitted. The purified STH was

stored at -20°C in buffer A with 2 mM dithiothreitol, with no detectable loss of activity over several weeks. Storage over several months resulted in great loss of activity, which could be reversed by incubation with fresh dithiothreitol. The N-terminal sequence of the recombinant enzyme was determined and was found to be identical to that determined for the enzyme purified from *P. fluorescens*.

Characterization of recombinant STH. The properties of the recombinant STH were compared to those reported for the enzyme from *P. aeruginosa*. The subunit M_r as determined by SDS-PAGE is consistent with that previously reported (13, 18).

1	50
mera_bacsr	MKKYRVNVQG MTCSCGEQHV AVALENMGAK AIEVDFFRGE AVFELPDVVK
51	100
mera_bacsr	VEDAKNAIAD ANYHPGEAEE FQSEQKTNLL KKYRLNVGEM TCTGCEEHIA
101	150
mera_bacsr	VALENAGAKG IEVDFFRGEA LFELPYDVDI DIAKTAITDA QYQPGAEIEI
151	200
sth_pseflAVYNY DVVV LGSGPA GEGAAMNAAG AGRKVAMVDS
udha_ecoliMGLVK QGARVAVIER
dldh_pseflSQKF DVVV IGAGCP GYVAAIRAAQ LGKLTACIEK
mera_bacsr	QVQSEKRTDV SLNDEGNVDY DYII IGSGGA AFSSAIEAVA LNAKVAMIE.
gshr_ecoliMTKHY DYIA IGSGSG GIASINRAAM YGQKCALIEA
nape_entfaM KVIV LGSGSG GYBAVEELLN LHPDAET...
201	250
sth_pseflRRQV GGNCTHLG TI PSKALRHSVR QIMQFNTNPM FRAI.GEPRW
udha_ecoliYQNV GGCCTHWG TI PSKALRHVS RIIEFNQNP L YSDH.SRLLR
dldh_psefl	YIGKEGKVAL GGTCLNVG CI PSKALLDSSY KYHEAKEAFK VHGIEAKGVT
mera_bacsrRGTV GGTCTNVGCV PSKTLRAGE INHLAKNNPF V.GLHTSASN
gshr_ecoliKEL GGTCTNVGCV PKKVMWHAQ IREAIHMYGP DYGFDTTINK
nape_entfaQWYKGD FISFLSCGMQ
251	300
sth_psefl	FSFPDVLKSA EKV...ISKQ VASR.TGYA RNRVDFFGT GSFADQETVE
udha_ecoli	SSPADILNHA DNV...INQQ TRMR.QGFYE RNHCEILQGN ARFVDEHTLA
dldh_psefl	IDVPAMVARK ANI...VKNL TGG.IATLTK ANGVTSFEGH GKLLANKQVE
mera_bacsr	VDLAPLVKQK NDL...VTM RNEKYVNLID DYGFELIKGE SKFVNENTVE
gshr_ecoli	FNWETLIASR TAY...IDRI HTS.YENVLG KNNVDVIGF ARFVDAKTFE
nape_entfa	LYLEGKVKDV NSVRVMTGCK MESRGVNVFS NTEITAIQP.KEHQVT
301	350
sth_psefl	VVCANGVVEK LVA.KHIIIA TGSRPYRPAD IDFHPRIYD SDTTL....
udha_ecoli	LDCPDGVSVE LTA.EKFVIA CGSRPYHPTD VDFTHPRIYD SDSLL....
dldh_psefl	VTGLDGKTVQ LEA.ENVIIA GSGRPVEIPP APLSDDIIVD STGAL....
mera_bacsr	VNGNQ....ITA.KRFLIA TGASSTAPNI PGLDEVDYLT STSL....
gshr_ecoli	VNG....ET TTA.DHILIA TGGRPSPHDI PGVEYG...ID SDGFF....
nape_entfa	VKDLVSGEER VENVYKLIIS PGAVPELDI PGKDLNLIY MRGRQWAKL
351	400
sth_psefl	...SLGHTPR KLIIY GAGVI GCEYASIFSG LGVLVELVDN RDQLLS.FLD
udha_ecoli	...SMHHEPR HVLII GAGVI GCEYASIFRG MDVKVDLINT RDRLLA.FLD
dldh_psefl	...EFQAVPK KLGVI GAGVI GLELGVSUAR LGAEVTVLEA LDKFLP.AYD
mera_bacsr	...ELKKVPN RLTVI SGSYI GLELGQLPHN LGSEVTLIQR SERLLK.EYD
gshr_ecoli	...ALPALPE RVAV GAGVI AVELAGVING LGAKTHLVR KHAPLR.SFD
nape_entfa	KQKTVDPEVN NVV IGSGYI GIEAAEAFAP AGKVTVIDI LDRPGLVYLD
401	450
sth_psefl	SEISQALSYP FSNNITVRH NEEYDRVEGL DNGVILHLK.SGKK
udha_ecoli	QEMSDLSYP FWNISGVVIRH NEEYKIEGK DDGVIMHLK.SGKK
dldh_psefl	BQIAKEALKV LTKQGLNIRL GA...RVT.A SEVKKKQVTV TPTDANGEQK
mera_bacsr	PEISEAITKA LTKQGLNIRL GATYERVEQD GDIKKHVHEI NGKKRIIEA.
gshr_ecoli	PMISETLVVE MNAEQPLQHT NAIKPAV...VKNTDGSL TL.ELEDGRS
nape_entfa	KEFTDVLTEE MEANNITAT GETVERVEGD GRVQKV....VTDKNA
451	500
sth_psefl	IKADALLWCN GRTGNTDKLG MENIGVKVNS RGQIEVDENY RTCVTNIYGA
udha_ecoli	LKADCLLYAN GRTGNTDSL LQNIQLETDG RGQLKVNMSY QTAQPHYVAV
dldh_psefl	ETFDKLIYAV GRPVTITDLL AADSGVTLDE RGFIYVDHDC KTSVPGVFAT
mera_bacsr	...EQLLIAT GRPPIQTSLN LHAAGVEVGS RGEIIVDDYL KTNISRIYSA
gshr_ecoli	ETVDCILWAI GREPANDIN LBAAGVKTNE KGYIVVDKYQ NTLNIGLIYAV
nape_entfa	YDADLVVAV GVRPNTAWL. ...KGTLELHP NGLIKTDEYM RTSEPDPVAV
501	550
sth_psefl	GDVI.....GWPSLA SAADHQGRSA AGSIV.DNGS WRY.VNDVPT
udha_ecoli	GDVI.....GYPSLA SAAYDQGRSA AQALVKGAT AHL.IEDIPT
dldh_psefl	GDVV.....RGAMLA HKASEEGVMV A.ERLAG.HK AQMNVDLIPS
mera_bacsr	GDVT.....PGPQFV YVAAYEGGLA ARNAIGG.LN QKVNLEVVPG
gshr_ecoli	GDNT.....GAVELT PVAVAAGRRR SERLFNNKPD EHLNDSINIPT
nape_entfa	GDATLIKYNP ADTEVNIALA TNARKQGRFA VKN.LEEPVK PFGVQGS
551	600
sth_psefl	GIYTIPEISS IKGNEHETLK AKVPYEV..G KAFFKSMARA QIAGEPQGM
udha_ecoli	GIYTIPEISS VGKTEQQLTA MKVPYEV..G RAQFKHLARA QIVGMNVGTL
dldh_psefl	VIYTHPEIAW VGKTEQTLKA E..GVEVNVG TFFPAASGRA MAANDTGLV
mera_bacsr	VTFPSPIAT VGLTEQQAKE K..GYEVKTS VLPDLAVPRA LVNRETTGVF
gshr_ecoli	VVFSPPIGT VGLTEQQAQRE QGDDQVKVY KSSFTAMYTA VTTTRQPCRM
nape_entfa	LAVFDYKFAS TGINE..VMA QXLGKETKAV TV.VEDYLM D FNPDKQKAWF
601	650
sth_psefl	KILFHRETLE VLGVHCFGYQ ASEIVHIGQA IMNQPGEQNT LKYFNTTTFN
udha_ecoli	KILFHRETKE ILGHCFCGER AAIEIHIGQA IMEQKGGNT IEFYNTTTFN
dldh_psefl	KVIADAKTKA VLGAHVHIGV AAELVQQAQI GME...FGTS ADELQGMVFS
mera_bacsr	KLIVADAKTLK VLGAHVHIGV AGDVIAATL AVK...PGLT VGDLDRETMAP
gshr_ecoli	KLVCVGSBEK IVCIHGIGFG MDEMLQGFV ALK...MGAT KDFDNTVIAI
nape_entfa	KLVDYDPETQ ILGAQLMS.. KADLTANINA ISLAIQAKMT IEDLAYADFF
651	682
sth_psefl	YPTMAEA...YRVAAYDGL NRLF.....
udha_ecoli	YPTMAEA...YRVAALNGL NRLF.....
dldh_psefl	HPTLSEA...LHEALAVN GHAIHIANRK KR
mera_bacsr	YLTMAEG...LKLAVLTFD KDVSKLSCCA G.
gshr_ecoli	HPTAAEE...FVTMR.....
nape_entfa	FQPAFKPWN INTAALEAV KQER.....

FIG. 3. Sequence alignment of STH and selected related enzymes. This alignment was generated by using the PILEUP program of the GCG package. The enzymes shown are as follows (with OWL database accession numbers in parentheses): sth_psefl, soluble transhydrogenase from *P. fluorescens* (this study); udha_ecoli, uncharacterized dehydrogenase from *E. coli* (P27306) (6); gshr_ecoli, glutathione reductase from *E. coli* (P06715) (5); dldh_psefl, dihydroliipoamide dehydrogenase from *P. fluorescens* (P14218) (1); mera_bacsr, mercuric ion reductase from *Bacillus* sp. strain RC607 (P16171) (17); nape_entfa, NADH

To determine whether the recombinant enzyme was capable of forming large polymers, samples were adsorbed to glow discharged carbon Formvar films from a solution of 1 mg of protein/ml, negatively stained with 1% (wt/vol) uranyl acetate, and examined by transmission electron microscopy with a Philips CM100 electron microscope operated at 80 kV. Long polymers of approximately 10 nm in diameter and in excess of 500 nm in length were observed (Fig. 2). This is consistent with previous reports (9) and shows unequivocally that only one type of subunit is required for polymer formation.

Sequence comparisons. The deduced amino acid sequence of STH was compared to other sequences in the OWL database. It was found that STH is highly similar in sequence to the flavoprotein disulfide oxidoreductases, particularly dihydroliipoamide dehydrogenases, mercuric ion reductases, glutathione reductases, and trypanothione reductases. The most similar sequence detected was that of an uncharacterized dehydrogenase from *E. coli* (6), encoded by the *udhA* gene, which showed 60% sequence identity and 77% similarity, as determined by the GCG GAP program (4). Various dihydroliipoamide dehydrogenases showed up to 31% identity and 52% similarity. A multiple sequence alignment of several related proteins is shown in Fig. 3. It is noteworthy that STH lacks one of the conserved cysteine residues which form the redox-active disulfide bond characteristic of this family of enzymes. A less closely related enzyme, the NADH peroxidase of *Enterococcus faecalis*, has been shown to possess an unusual redox center consisting of a single stabilized cysteine-sulfenic acid residue (11); however, it seems likely that no redox center other than the flavin is required in STH, which appears to operate by a simple ping-pong mechanism (19).

The recombinant STH did not show significant reduction of liipoamide (<0.2 U/mg) in an assay system consisting of 0.2 mM NADPH or NADH and 0.2 mM liipoamide in 50 mM Tris-HCl buffer (pH 7.0) at 30°C. Under these conditions, dihydroliipoamide dehydrogenase from bovine intestinal mucosa (Sigma) displayed vigorous activity (100 U/mg) with NADH but no significant activity (<0.1 U/mg) with NADPH. Lack of dihydroliipoamide dehydrogenase activity in STH is consistent with earlier reports (2).

Glutathione reductases, trypanothione reductases, and mercuric ion reductases are active as homodimers (21). Dihydroliipoamide dehydrogenases form a part of several multienzyme complexes, including pyruvate dehydrogenase and α -ketoglutarate dehydrogenase (E3 component), branched-chain oxoacid dehydrogenase, and the glycine cleavage system (L-chain). In these complexes they form homodimers which interact with other components. The enzymes of this family consist of three domains: an N-terminal flavin-binding domain, a central NAD(P)-binding domain, and a C-terminal dimerization domain. The N-terminal and central domains show considerable similarity and may have evolved through gene duplication (10). The relationship of STH to these enzymes casts no obvious light upon the polymerization of STH or its apparent binding to the catalytic site (7). Conceivably, certain of the binding sites in the active 30-subunit form act as regulatory sites and others function as active sites. Such a mechanism was earlier proposed to account for incomplete reduction of flavin by reduced cofactors (2). In this regard it is interesting that negative co-

peroxidase from *E. faecalis* (P37062) (12). Indicated in bold type are the redox-active cysteine residues and the Rossman fold Gly-X-Gly-X-X-(Gly/Ala) motifs forming the flavin adenine dinucleotide and NAD(P) binding sites (10, 20).

operativity in cofactor binding has been reported for dihydro-lipoamide dehydrogenase and glutathione reductase (21). We hope to address these questions through structural studies of the recombinant enzyme. The availability of large amounts of STH will also enable us to investigate the use of this enzyme for cofactor cycling in biotransformation processes dependent on both NAD and NADP.

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